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Funnel ratchets in biology at low Reynolds number: choanotaxis

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We present an example of a ratchet phenomena in biology consisting of swimming bacteria in an enclosed structure separated by a wall of funnels. The funnels serve to concentrate bacteria that are motile, in spite of the fact that the motion occurs at low Reynolds numbers and would be expected to be time reversible invariant. We discuss some of the basic ideas which underly the phenomena including spatial and temporal coherence of the motion of bacteria and interaction of the bacteria with surfaces.

Keywords: ratchet; biology; bacteria; low Reynolds number; reversible; phase space; time dependence

1. Introduction

Ratchet phenomena occur in venues as distinct as interacting quantum systems [1] to the device that tightens your tennis net. A ratchet is characterized by the irreversible movement of an object in space between positions of equivalent potential energy. Typically time-irreversible ratchet phenomena involve the periodic changing of potential surfaces and diffusive movements during the change of the potential surfaces, a phenomena called brownian ratchets [2]. The diffusive motion (or quantum dephasing in the case of a quantum system) makes the motion irreversible and a ratchet motion ensues. Conservation of energy is ensured by the work done by the moving potential on the object that is being ratcheted. However, not all ratchet phenomena necessarily involve the time varying movement of a potential surface, and disturbingly would seem to only involve diffusion and asymmetric but fixed boundaries, calling into question the the 2nd 'Law' of thermodynamics and invoking the specter of Maxwell's Demon [1]. Maxwell's Demon was an imaginary agent who let molecules through a gate depending on their speed, fast to the right of the gate, slow to the left. In this way the Demon, if it existed, could without doing any work create a temperature difference between the two chambers in violation of the 2nd Law of Thermodynamics. Another form of the Demon could simply open a gate when a particle approached and therefore concentrate particles on one side without doing work.

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While a Maxwell Demon cannot in fact concentrate objects without doing work, although how that 'work' is done can be very subtle and leads into the energy cost of computation, we can do the inverse action: we can make an opening which is asymmetric to the right and to the left side of two partitions and let self-propelled bacteria interact with the asymmetric opening. We call this 'Choanotaxis', after the Greek word for 'funnel', choano. Self-propelled organisms in a choanotactic medium are allowed to 'violate' the 2nd Law since they do work on the system, and they can in fact act as Maxwell Demons as we show here using microfabricated asymmetric openings to concentrate swimming bacteria. However, exactly *how* this happens and choice of the correct design parameters is rather controversial, and we will try to wrestle with this issue.

2. The toy membrane

About 30 years ago one of the authors (RA) was sitting in his office as an Assistant Professor when the theorist professor he had come to work for came to his office and remarked about a strange conversation he had had with a visitor [3]. The visitor had a device very similar to the one shown in Figure 1(*a*), which was simply a thin rectangular enclosure with a wall of funnels separating two halves of the enclosure. If an initially random distribution of rod-like objects was placed in the rectangle such that equal

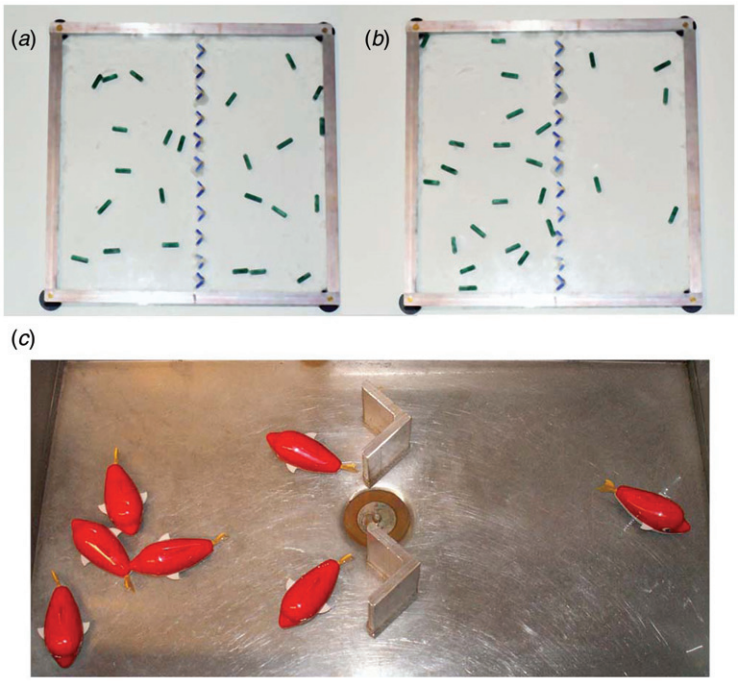

Figure 1. (*a*) The results of shaking rods with a funnel barrier: before shaking horizontally by hand. (*b*) After 30 s of vigorous shaking. (*c*) Self-propelled bathtub toys after 30 s of motion. (The color version of this figure is included in the online version of the journal.)

numbers of the rods was on both sides of the funnel, and if the device was shaken vigorously back and forth (more on this later!) in a few minutes the distribution of the objects would now become very much asymmetrical: most of the rods ended up on half of the rectangle facing the narrow side of the funnels, as shown in Figure 1(*b*). The idea proposed by the visitor was that a membrane consisting of a surface of such funnels made on a nano-scale could concentrate molecules using just thermal motion! Of course, this would violate the 2nd Law of Thermodynamics and in fact act as a perpetual motion machine, since the chemical potential $\mu = k_B T \log[\rho_{left}/\rho_{right}]$ created by an imbalance in the densities of particles ρ_{left} and ρ_{right} of the funnel wall could be used as a free energy source extracted from an isothermal bath. You might not be convinced that shaking the rods is really an example of what molecules moving in solution might do, as a further example in Figure 1 we show what happens to self-propelled rubber duckies swimming in a bathtub with funnel walls: they too are concentrated on the closed side of the funnel cones.

Although the concentration of the rods on one side of the device when shaken by hand or the self-propelled bathtub toys (ideally rubber duckies but in our case toy dolphins) would also seem to violate the 2nd Law, in fact the result although intriguing does not cause any problems in statistical mechanics because the full Navier–Stokes equation is not invariant with time reversal at high Reynolds number motion where the inertial $\partial \boldsymbol{v}/\partial t$ term cannot be neglected:

$$m\left[\frac{\partial \boldsymbol{v}}{\partial t} + \boldsymbol{v}(\nabla \cdot \boldsymbol{v})\right] = -\zeta \boldsymbol{v} + \nabla P, \tag{1}$$

where m is the mass of the object, $\boldsymbol{v}$ is the velocity of the object, ζ is the coefficient of friction and P is the local pressure.

At low Reynold's numbers, however, the situation is different, since the inertial $\partial \boldsymbol{v}/\partial t$ term is small compared to the viscous damping term and the spatial derivative of $\boldsymbol{v}(\nabla \cdot \boldsymbol{v})$ turns the Navier–Stokes equation to:

$$m\nabla^2 \boldsymbol{v} = -\zeta \boldsymbol{v}. \tag{2}$$

The Navier–Stokes equation at low Re has the same dynamic behavior if time is reversed and if the forces are reversed in sign: that is the displacement of particles moves back to where they were originally at time $t = 0$ if after a time $t_{reverse}$ the forces are reversed in sign, which is the same as running time backwards. Of course, if diffusion is allowed to occur then this reversibility is violated and we have exploited this fact in the separation of particles using a combination of asymmetric obstacles, driven flow and diffusion in what is called a 'brownian ratchet' [4,5]. However, motile biological cells can swim and drive energy into a system. In particular, *Escherichia coli* (*E. coli*) bacterial cells are self-propelled via motors which spin a handed set of flaggelae [6]; these flagellae when rotated in the same sense as the helical pitch (left-handed) mesh together and screw the bacteria through the liquid at low Reynold's numbers [7]. The motion of the center of mass of the *E. coli* resembles a random walk [8], but is not brownian motion, brownian motion has no length scale and the concept of a 'run length' does not occur for true brownian motion [9].

Swimming *E. coli* alternate running (continuous translation along a straight line for a distance l_T) and tumbling (random change in orientation without translation) cycles.

All flagella have the same handedness, and during runs (when they rotate counter-clockwise) they assemble into a bundle. Reversing the rotation direction of the helical flagella doesn't result in a backward motion of the bacteria. Instead the flagella jam and the bundle disintegrates. This results in a random change in the swimming direction and introduces a time-irreversible component in the swimming process. The bacteria are self-propelled but have time-irreversible motion in spite of their random-like walk movement. Moreover, the time-reversible form of the Navier–Stokes Equation (1) doesn't account for phenomena at the liquid–solid interface, such as inelastic collisions of an object with the wall. Thus, there is a problem with a pure hydrodynamic approach. Quoting from one of our colleagues: '*Suppose I run your movie forward – the bugs are evenly distributed, then go to one side and then assume a random array of swimmers. Now instead I start with a random set of swimmers on the back side of the funnel I run the movie and they should go back to the up side of the funnels. They don't. So that's what bugged me. What's the answer?*' [14]. In other words, given that we are at low *Re* the bacterial motion should be reversible and it isn't. The question is: when bacteria are exposed to a funnel wall such as is shown in Figure 1 will they also concentrate like the bath-tub toys of high Reynold's motion?

3. The bacterial funnel

We showed in a recent paper [11] that swimming bacteria can in fact 'self' concentrate in a funnel structure, although as we will show now the design of the funnel and the length scale of the device seems to be rather critical. Figure 2 shows the basic structure of these ratchet devices, and Figure 3 shows the basic phenomena, over a period of several hours motile bacteria (but NOT non-motile bacteria) concentrate themselves across a funnel wall.

The critical quantity here is the effective step length l_T that the bacteria make between tumbles: note that $l_T = 0$ for true brownian motion [9], and that this distance represents a deterministic length not governed by equilibrium thermodynamics. If you like, you can view it as a dephasing distance, or in laser terminology the 'coherence length' of the motion, although here we are not talking about the phase of the wave function but rather the straight line path. Let $A(t) = [\rho_L(t)/\rho_R(t)]$ be the observed anisotropy versus time of the bacterial densities, where ρ_L is the average bacterial densities on the left and ρ_R is the average bacterial densities on the right side of the funnel barrier. If we assume that the

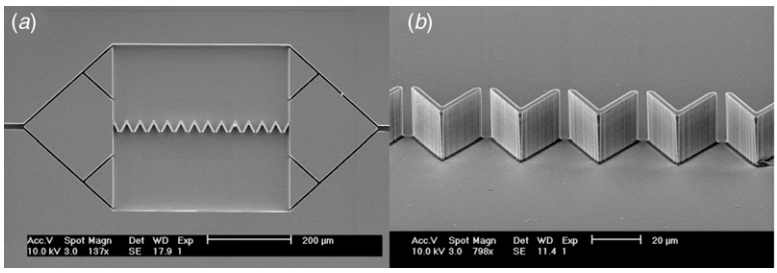

Figure 2. SEM pictures of the microfabricated funnel wall. (*a*) Top view. (*b*) Side view of the 20 µm etch depth.

bacteria execute a random walk with step length l_T of 50 μm, which is given by their mean swimming speed v_S of 10 μm s^{-1} and their observed mean time between tumbles t_{tumble} of 5 s, the radius of gyration R_g for N steps, where $N = t/5$, is given by:

$$R_g(t) = \left(\frac{N}{6}\right)^{1/2} l_p \sim \left(\frac{t}{30}\right)^{1/2} l_p. \tag{3}$$

Thus, the time t_c for a bacterium to explore the 200 μm depth of one of the confining regions is roughly 10 min. We would thus expect initially that the $A(t)$ would increase exponentially in time with a time constant τ of roughly 10 min. However, $A(t)$ cannot increase indefinitely because of the chance for leakage of the bacteria back into the 'wrong' side. The experiments suggest that the shape of the separating wall and the interaction of bacteria with this wall has a major role in forming the inhomogeneous cell distribution. Our observations show that bacteria reaching a wall during a run will continue swimming along the wall from purely hydrodynamic shear forces due to the termination of the flow field by the boundary condition of no-slip. This wall effect term is not present in Equation (2), when it is included the symmetry breaking of the angled wall allows the observed concentration to occur by movement of the bacteria along the funnel walls.

Thus, because of this tendency the effective cross-sectional opening for bacterial traversal is different for the two sides, in our case the cross-sectional ratio $A(t=\infty) = W_L/W_R$. Our standard geometry consists of 13 funnels with 27 μm long sides and a 60° apex. We can give a crude estimate for the ratio of cell densities on the two side of the funnel wall. The gaps G are 3.8 μm. For this we have to consider the angle under which a bacterium has to approach the funnels to get through and the location along the wall towards which the bacterium is swimming. On the restricted side the cell may approach from any direction (a 180° range), but it has to reach the wall exactly at the gap (the probability of this is given by the total gap size divided by the wall length). This gives $W_L = 180/360 * 13 * 3.8/400 = 0.062$. However, from the open side of the wall the cells may approach in a 120° range to be diverted towards the gap by the funnel wall. In this case it doesn't matter where they hit the wall, they are directed towards the gap given they approach from a suitable angle. Here $W_R = 120/360 = 0.33$. We then predict that the equilibrium density ratio $A(t=\infty)$ for motile bacteria by detailed balance are given by:

$$A(t = \infty) \sim W_R/W_L = 0.33/0.062 = 5.3 \tag{4}$$

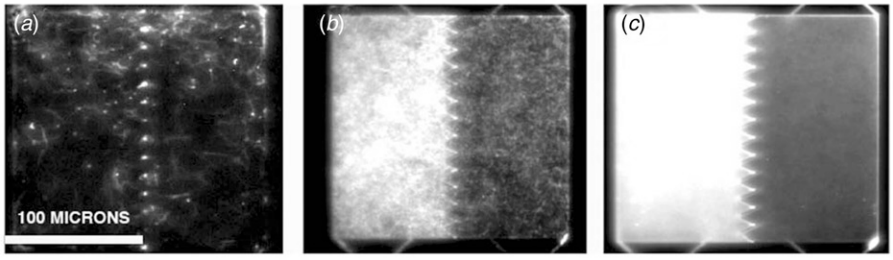

Figure 3. Bacterial density versus time in a funnel array. (*a*) Initial injected bacterial density. Scale bar = 100 μm. (*b*) Density after 24 h, single frame exposure. (*c*) Density after 24 h, 100 frame average.

and the time-dependence of $A(t)$ is given by:

$$A(t) = \frac{1 - [(W_L - W_R)/(W_L + W_R)]x}{1 + [(W_L - W_R)/(W_L + W_R)]x}; \quad x = [\exp(-t/\tau) - 1], \tag{5}$$

where $\tau = D/h(W_R + W_L)$ and D is the effective 'diffusion coefficient' of the swimming bacteria, $D = v_S^2 t_{\text{tumble}}$.

Figure 4 shows the clear buildup of the cell density anisotropy $A(t)$ versus time over a period of ~300 min. We also show in Figure 4 a fit of Equation (5) to the data, the best fit for τ is 80±5 min and the best fit for W_R/W_L is 3.1±0.1, yielding an equilibrium concentration ratio of 3:1, but not the expected 5:1. Why?

4. The trouble with scaling

The data above came from a particular set of parameters and length scales. Do the length scales matter? One can do two things with the design: simply scale it up by some factor γ keeping $A(t = \infty)$ constant at 5.3, or keep the box the same and simply change the gap between the apex of the funnel from 3.8 to 10 μm, thus making $A(t = \infty) \sim 1.5$.

First, we consider what happens when the gap is simply opened up, lowering $A = W_R/W_L$ to 2. We would expect to still see population imbalance, with a 2/1 ratio. But this doesn't happen. A repeat of the experiment with wider gaps is shown in Figure 5, and the measured ratio of the populations on either side drops to 1.2/1, barely significant.

A more telling experiment was to simply scale the device up by a factor of 10 so that W_R/W_L is still 5.3, but now the gap is 38 μm wide instead of 3.8 μm. Figure 6 presents the results of that experiment: the measured ratio A between the two sides drops to 1.45/1 from 3/1, so simply scaling the size of the funnels definitely has an effect: the wider the funnels, the smaller the pumping. Obviously, there is more to the pumping effect than simple geometrical factors.

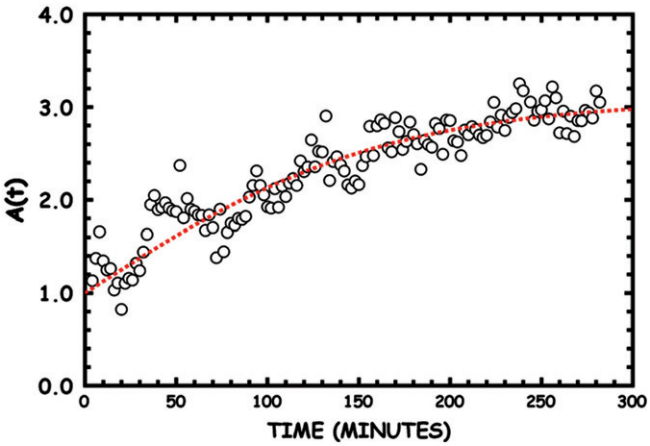

Figure 4. Open circles: measured density anisotropy, dashed red line: fit of (5) to the density anisotropy. (The color version of this figure is included in the online version of the journal.)

5. A partial resolution to the paradox

The basic mechanism by which the imbalance of the bacterial population grows with time has been worked out in a beautiful paper by Wan et al. [12]. Here is the basic idea: the funnels work as long as the swimmers move out of thermal equilibrium, that is, in

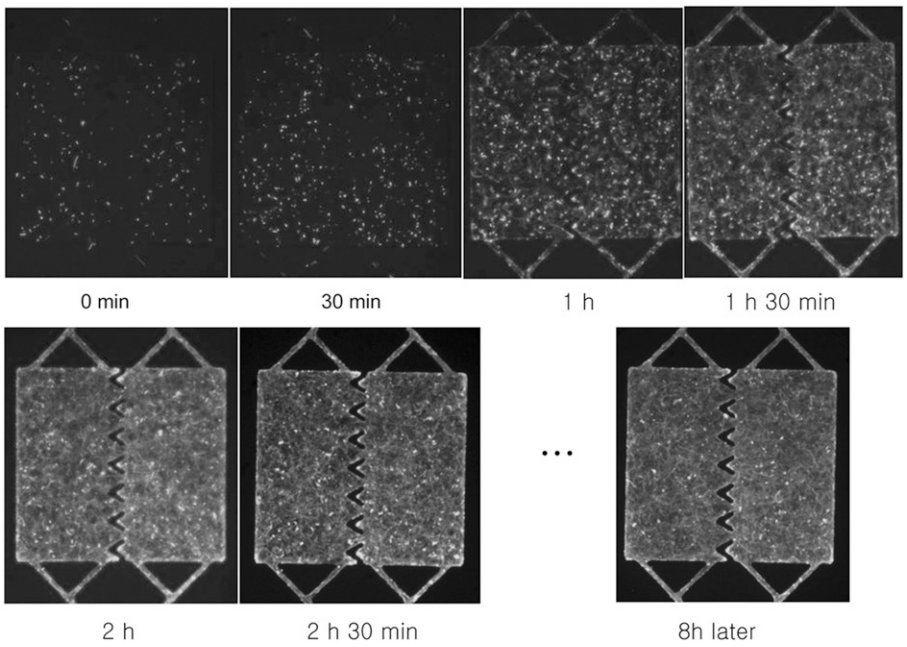

Figure 5. *E. coli* population in equilibrium when the gap is 10 μm wide.

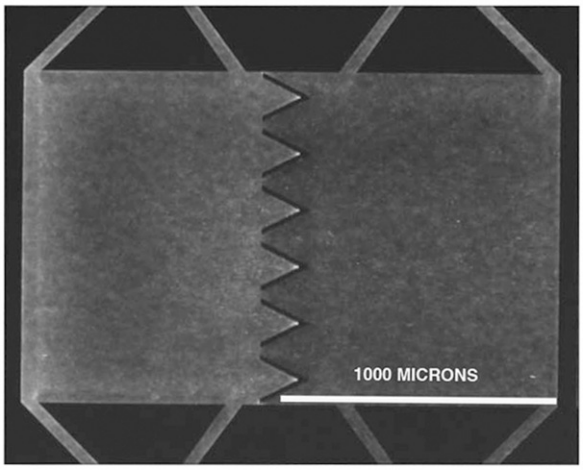

Figure 6. *E. coli* population in equilibrium when the device is scaled to ×10 the length scale of the device in Figure 3.

a straight line at constant speed. Once the object tumbles and loses its sense of direction, the tumbling event acts as a thermalization event, or in the parlance of modern optics the loss of coherence. Thus, we shouldn't expect that for a truly brownian motion that the funnel wall can serve to concentrate objects on one side, and the critical number at which rectification can occur is the ratio of the mean straight line path between thermalization events L_p and the gap width of the funnel G. If $G \gg L_p$, we expect no pumping due to swimmers. This is of course what we observe for our bacteria: assuming that the mean straight line path between tumbles is approximately 50 μm [8] we would expect with scaling of the device or opening of the gap beyond 50 μm that the ratcheting effect decreases, as we have observed.

However, we had another failure in the application of this device to concentrate other moving organisms that does not quite seem to fit this picture. *Caenorhabditis elegans* is a model organism for multicellular eukaryotes. The entire genome of this organism was sequenced and all of its cells have been mapped as well [15]. It is an ideal organism for doing comparative studies in behavior since their behavior can be inferred to be similar to what would occur in other multicellular organisms. *C. elegans* move via a sinusoidal moving wave which is launched along the length of the organism as it moves on surfaces, a very different mechanism than is used by *E. coli*. This idea was then applied to *C. elegans* to see if concentration could be achieved with them as well. The funnel structure consisted of a series of 12 funnels with gaps of 200 μm between each funnel (Figure 7). If the worms followed the walls after a collision as we claim the *E. coli* do, that is, the effect is purely due to organism–wall interactions, then worms would be funneled from the right side to the left side, and not from the left to the right side.

In this experiment the plates were observed underneath a stereomicroscope to determine if there were eggs on the plate. Three plates with eggs were rinsed with a combined amount of 3.5 ml of sterile deionized water and collected in a centrifuge tube. In another tube, 0.5 ml of 5 M sodium hydroxide solution (NaOH) and 1.0 ml of bleach were mixed and then added to the centrifuge tube containing the worms. This tube was shaken every two minutes for ten minutes. The mixture was centrifuged at 1300 g for one minute and decanted until 0.1 ml was left. Sterile water was added to the tube to achieve

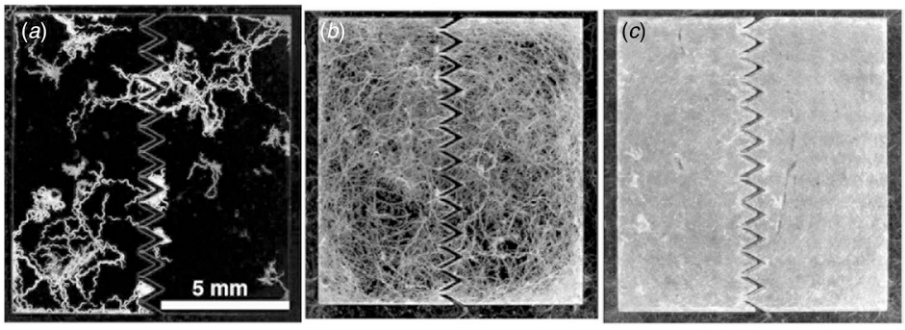

Figure 7. *C. elegans* migrating through a funnel array. The scale bar shows the dimension of the box. (*a*) Single frame picture of approximately 100 worms. (*b*) 100 frames, with one frame taken every 5 s. (*c*) Superposition of all frames taken over 10 h at 1 frame/5 s.

a level of 5 ml, centrifuged, and decanted. The remaining liquid was diluted with NGM buffer and delivered to a plate containing *E. coli*. Sterilized deionized water in aliquots of 200 μl was used to rinse a synchronized plate of worms. The water containing worms was transferred to a tube and centrifuged using a tabletop centrifuge. The concentrated worms at the bottom of the tube were transferred to an unseeded NGM plate.

Figure 7 shows a set of increasingly larger number of superimposed frames of approximately 100 *C. elegans* crawling over the surface of the device. Since the typical length of these nematodes is approximately 1 mm the tumbling arguments of Wan et al. [12] cannot apply and one would think that perhaps the *C. elegans* would also show population rectifucation. However, it is clear that there is little enhancement due to the funnels as the nematodes crawl over the surface of the structure over a period of 10 h.

Perhaps these funnel experiments – Choanotaxis experiments – are a bit strange. In the case of bacteria, the observed ratcheting of the populations seems understood, in the case of the *C. elegans* perhaps not. We think that these choanotaxis experiments using asymmetric structures, combined with physical analysis using statistical mechanics, could be used as physical probes of biological adaptation and evolution, to judge how organisms can learn to sense and adapt to their environments in ways we can understand from a physics perspective.

Acknowledgements

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